

ORIGINAL ARTICLE

Effect of diets rich in either saturated fat or n-6 polyunsaturated fatty acids and supplemented with long-chain n-3 polyunsaturated fatty acids on plasma lipoprotein profiles

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BACKGROUND/OBJECTIVES: Abnormalities in lipoprotein profiles (size, distribution and concentration) play an important role in the pathobiology of atherosclerosis and coronary artery disease. Dietary fat, among other factors, has been demonstrated to modulate lipoprotein profiles. We aimed to investigate if background dietary fat (saturated, SFA versus omega-6 polyunsaturated fatty acids, n-6PUFA) was a determinant of the effects of LCn-3PUFA supplementation on lipoprotein profiles.

SUBJECTS/METHODS: A randomized controlled clinical intervention trial in a parallel design was conducted. Healthy subjects ($n=26$) were supplemented with 400 mg eicosapentaenoic acid plus 2000 mg docosahexaenoic acid daily and randomized to consume diets rich in either SFA or n-6PUFA for a period of 6 weeks. Blood samples, collected at baseline and after 6 weeks of intervention, were assessed for plasma lipoprotein profiles (lipoprotein size, concentration and distribution in subclasses) determined using nuclear magnetic resonance spectroscopy.

RESULTS: Study participants receiving the SFA or the n-6PUFA enriched diets consumed similar percentage energy from fat (41 and 42% respectively, $P=0.681$). However, subjects on the SFA diet consumed 50% more energy as saturated fat and 77% less as linoleic acid than those consuming the n-6PUFA diet ($P<0.001$). The diets rich in SFA and n-6PUFA reduced the concentration of total very-low-density lipoprotein (VLDL) particles ($P<0.001$, both), and their subclasses and increased VLDL ($P=0.042$ and $P=0.007$, respectively) and LDL ($P=0.030$ and 0.027 , respectively) particle size. In addition, plasma triglyceride concentration was significantly reduced by LCn-3PUFA supplementation irrespective of the dietary fat.

CONCLUSIONS: LCn-3PUFA modulated lipoprotein profiles in a similar fashion when supplemented in diets rich in either SFA or n-6PUFA.

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INTRODUCTION

Abnormalities in lipoprotein profiles have been associated with coronary heart disease risk in men¹ and women.^{2–5} Evidence suggests that coronary heart disease risk is increased by larger very-low-density lipoprotein (VLDL) particle sizes, smaller high-density lipoprotein (HDL) and LDL particle sizes, and increased concentration of small LDL and large VLDL particles; although the ideal lipoprotein profiles for the prevention of coronary heart disease may vary according to age, gender and health status.^{1,6} A variety of factors can modulate lipoprotein profiles, including dietary habits⁷ or physical activity.⁸

Long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA), despite some controversy,⁹ are well known for their health benefits, particularly in the prevention of coronary heart disease, due to their capacity to help manage hyperlipidaemia,¹⁰ inflammation¹¹ and platelet aggregation,^{11,12} as well as their potential to modulate lipoprotein profiles. Dietary supplementation with LCn-3PUFA has been shown to decrease the concentration of small HDL particles,¹³ and increase the concentration of large HDL particles^{13–15} and the average HDL particle size,¹⁶ although change in HDL particle size has not been observed in all

studies.^{15,17} LCn-3PUFA supplementation has also been shown to decrease VLDL particle size^{15,18} and large and medium VLDL particle concentration.¹⁴ However, the effect of LCn-3PUFA supplementation on the LDL profile is heterogeneous. Some authors suggest an increase in LDL particle size,^{15,19,20} while others have not observed change in LDL size^{17,21–23} or have even observed a decrease in particle size.¹³ The literature also suggests an increase in the concentration of medium and large LDL particles, and a decrease in small particles, despite no change in the average size of the particles.^{14,15,22}

In all the above mentioned studies involving LCn-3PUFA supplementation and lipoprotein profiles, little attention has been given to the other components of the diet, especially the amount and type of the fats consumed. The human diet comprises a mixture of fats and even after supplementation and increase in seafood consumption, LCn-3PUFA remains a minor proportion of the total fat consumed. We have recently demonstrated that LCn-3PUFA are incorporated in plasma and erythrocytes to a greater extent when consumed with a diet rich in saturated fats compared to a diet rich in n-6PUFA.²⁴ The aim of this study was to examine whether the background

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dietary fat-induced changes in lipoprotein concentration, size and subclass distribution are modulated by co-supplementation with LCn-3PUFA.

SUBJECTS AND METHODS

Subjects

Healthy adults aged between 18 and 65 years were recruited. Subjects were excluded if they were using lipid-lowering drugs (for example, statins) or fish oil supplements; regularly consuming two or more fish meals per week; had any history of stroke, congestive heart failure, coronary artery bypass graft, myocardial infarction, or atherosclerotic cardiovascular disease; had diabetes, liver or gastrointestinal disease; were smokers; were pregnant or breastfeeding.

Study design

This randomized, parallel design, dietary intervention trial has been previously described.²⁴ In summary, subjects were randomized using computer-generated random tables to diets supplemented with LCn-3PUFA and either enriched with saturated fatty acids (SFA+LCn-3PUFA diet) or n-6PUFA (n-6PUFA+LCn-3PUFA diet). Subjects in the SFA+LCn-3PUFA diet group consumed daily 24 g of butter, 40 g of white chocolate (30 g fat, 20.9 g saturated fat) and 4 × 1 g fish oil capsules (100 mg eicosapentaenoic acid (EPA) and 500 mg docosahexaenoic acid (DHA) each (EPAX 1050TG, Norway)). Participants were also encouraged to consume foods containing SFA, while reducing n-6PUFA intake. Subjects on the n-6PUFA+LCn-3PUFA diet received 20 g of margarine, 42 g of sunflower seeds (30 g fat, 20 g n-6PUFA) daily and 4 × 1 g fish oil capsules. They were also guided on how to increase consumption of foods containing n-6PUFA while reducing SFA intake. All subjects were advised to maintain other aspect of their habitual diet and their usual physical activity status. Before starting and on their last week on the intervention, subjects completed a 3-day food record (2 week days and 1 weekend day), a physical activity questionnaire and a medical questionnaire, which included current medications and supplements, illnesses and alcohol consumption. FoodWorks 7 Professional (Xyris software, Brisbane, QLD, Australia) was used to assess the food records. A fasting blood sample was collected in EDTA vacutainers at baseline and following 6 weeks of dietary supplementation, and plasma lipoprotein profiles were determined. Blood samples were also analysed for fatty acids as previously reported.²⁴ Subjects were asked to refrain from vigorous exercise and alcohol consumption for 24 h prior to the study day and fasted for at least 10 h prior to each blood collection. The study was carried out in accordance with the guidelines provided in the Declaration of Helsinki. The study protocol was approved by the University of Newcastle's Human Research Ethics Committee (protocol H-2012-0117) and registered with the Australia New Zealand Trial registry (identification number ACTRN12613000962730, <http://www.anzctr.org.au/>). All participants gave written consent prior to participation.

Adherence to the dietary supplementation was determined by counting the left-over LCn-3PUFA capsules and interventional food products provided (butter, white chocolate, margarine and sunflower seeds), querying subjects about their food intake, assessing subjects' dietary records and analysis of plasma fatty acid composition. None of the participants reported any signs of intolerance to the supplements or the study foods.

Plasma lipoprotein profiles

Blood samples were centrifuged (1000 g × 15 min at 4 °C) immediately after collection to separate plasma from erythrocytes and the fractions were stored at -80 °C until analysed.

Plasma samples were analysed for lipoprotein profiles using nuclear magnetic resonance spectroscopy (NMR) at Biosfer Teslab (Reus, Spain). The detailed methods for the determination of lipoprotein profiles (size, concentration and subclass distribution) has been reported previously.²⁵ Lipid volumes were calculated by using common conversion factors to convert concentration units into volume units.²⁶ The size range for different lipoprotein subclasses corresponded to: large VLDL, 68.5–95.9 nm; medium VLDL, 47–68.5 nm; small VLDL, 32.5–47 nm; very large LDL, 24–32.5 nm; medium-large LDL, 20.5–24 nm; small LDL, 17.5–20.5 nm; very large HDL, 10.5–13.5 nm; medium-large HDL, 8.5–10.5 nm; and small HDL, 7.5–8.5 nm.

Statistical analysis

This study was primarily designed to assess blood lipid levels, discussed in Dias *et al.*²⁴ Thirteen subjects were necessary to cause a 30% (0.45 mmol/l) decrease in fasting plasma triglycerides with 0.387 mmol/l of s.d., 0.05 of significance level and 80% power.

All data are presented as median and interquartile range, and non-parametric statistical tests were employed on the analysis. Kruskal–Wallis test was used for baseline comparisons. Data at baseline and 6 weeks were compared within groups using Wilcoxon signed-rank test and data on change were compared between groups using Kruskal–Wallis test. Statistical analysis were performed using Mat Lab R2010a (MathWorks, Natick, MA, USA) and *P*-values <0.05 were considered statistically significant.

RESULTS

From the 33 subjects who completed the intervention period (18 on the SFA+LCn-3PUFA diet and 15 on the n-6PUFA+LCn-3PUFA diet), 7 failed to adhere to the intervention diets or the supplementation (compliance <80%) and were, therefore, excluded from the data analysis (Figure 1). Twenty six healthy subjects were then included in the statistical analysis, 14 subjects on the SFA+LCn-3PUFA diet (3 males and 11 females) and 12 on the n-6PUFA+LCn-3PUFA diet (3 males and 9 females).

The concentration of LCn-3PUFA in the fish oil capsules supplemented, measured using gas chromatography,²⁷ was >70% total LCn-3PUFA (14.3% EPA and 58.8% DHA) as previously described.²⁴

Subjects' general characteristics, dietary intake, and plasma and erythrocyte fatty acid composition have been discussed previously; the full discussion and other supporting materials are available in Dias *et al.*²⁴ and summary tables are also available in the Supplementary Material (Tables SM.1–SM.3). In summary, subjects on the SFA+LCn-3PUFA and the n-6PUFA+LCn-3PUFA diet did not differ significantly in age (40.0 (15.0) and 43.5 (27.5), respectively), body mass index (22.2 (4.9) and 22.7 (4.3), respectively), percentage body fat (26.2 (18.5) and 26.4 (10.6), respectively) and blood lipids at baseline. As expected participants randomized to the SFA+LCn-3PUFA diet increased saturated fat and decreased linoleic acid intake (*P* < 0.01, both), while participants randomized to the n-6PUFA+LCn-3PUFA diet increased linoleic acid and decreased saturated fat intake (*P* < 0.01, both) compared to baseline values. Changes in dietary protein, total fat, carbohydrates and cholesterol did not differ significantly between dietary groups. There were no significant differences in plasma fatty acids between the two groups at baseline, except for percentage DHA that was significantly greater in the plasma of subjects randomized to the SFA+LCn-3PUFA diet. Changes in dietary patterns and plasma fatty acids suggest that participants complied with the study protocol. Change in plasma LCn-3PUFA (including EPA, docosapentaenoic acid and DHA) was significantly greater (*P* < 0.05) after the consumption of the SFA+LCn-3PUFA diet compared to the n-6PUFA+LCn-3PUFA diet. There was also a significant increase in percentage linoleic acid and a concomitant decrease in percentage arachidonic acid (AA) after the consumption of n-6PUFA+LCn-3PUFA diet, while no significant change in linoleic acid and AA was observed after the SFA+LCn-3PUFA diet.

Lipoprotein profiles at baseline were not significantly different between groups (Tables 1 and 2). Both diets decreased cholesterol and triglyceride concentrations in VLDL particles (n-SFA+LCn-3PUFA diet, *P* < 0.001; n-6PUFA+LCn-3PUFA diet *P* = 0.002 and *P* < 0.001, respectively). After the n-6PUFA+LCn-3PUFA diet, there was a significant decrease in cholesterol and triglyceride levels in intermediate-density lipoprotein particles (*P* = 0.001 for both). After the SFA+LCn-3PUFA, there was a significant increase in the concentration of LDL triglycerides (*P* = 0.002). In addition, subjects on the SFA+LCn-3PUFA diet presented significantly higher triglyceride levels in

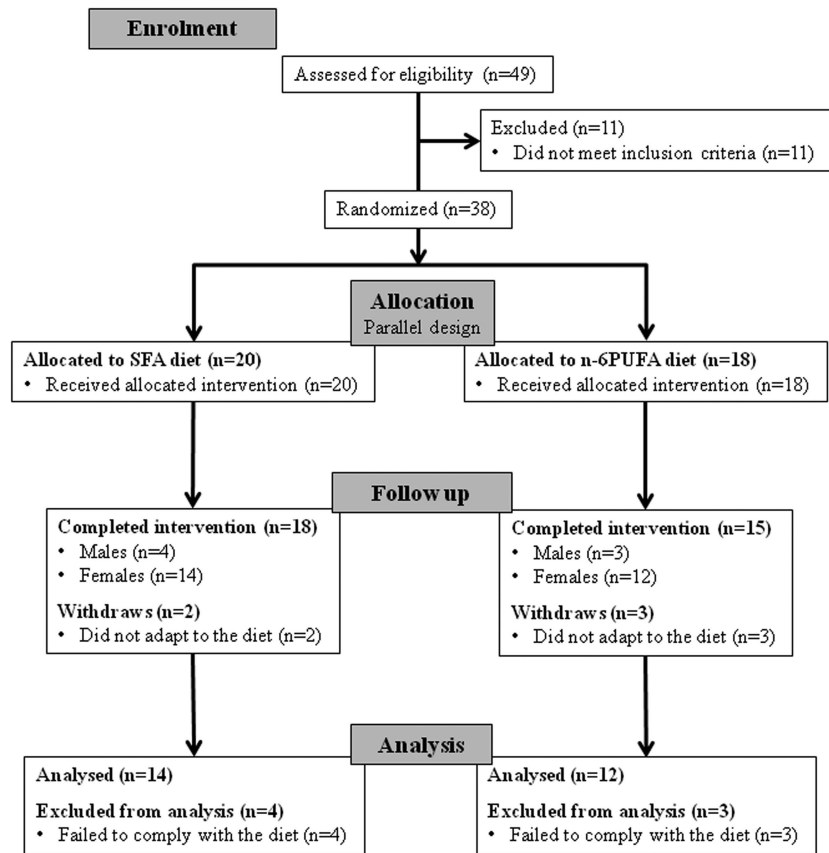


Figure 1. Enrolment schedule for the interventional trial.

Table 1. Plasma lipid concentrations of subjects at pre and post intervention, measured using nuclear magnetic resonance spectroscopy

mmol/l		Pre-intervention	Post-intervention	Δ Change	P-value ^a
VLDL-C	SFA+LCn-3PUFA	0.12 (0.12)	0.02 (0.02)	-0.05 (0.08)^b	0.129
	n-6PUFA+LCn-3PUFA	0.14 (0.15)	0.02 (0.07)	-0.11 (0.15)^b	
IDL-C	SFA+LCn-3PUFA	0.09 (0.06)	0.09 (0.06)	-0.01 (0.06)	0.076
	n-6PUFA+LCn-3PUFA	0.09 (0.07)	0.04 (0.07)	-0.04 (0.08)^b	
LDL-C	SFA+LCn-3PUFA	2.70 (0.51)	2.71 (0.59)	0.13 (0.57)	0.425
	n-6PUFA+LCn-3PUFA	2.50 (0.72)	2.43 (0.29)	0.01 (0.47)	
HDL-C	SFA+LCn-3PUFA	1.39 (0.40)	1.55 (0.23)	0.07 (0.32)	0.857
	n-6PUFA+LCn-3PUFA	1.24 (0.45)	1.27 (0.58)	0.08 (0.17)^b	
Total C	SFA+LCn-3PUFA	4.23 (0.78)	4.57 (1.09)	0.13 (0.67)	0.355
	n-6PUFA+LCn-3PUFA	4.01 (0.93)	3.99 (0.57)	-0.06 (0.49)	
VLDL-TG	SFA+LCn-3PUFA	0.44 (0.30)	0.29 (0.27)	-0.17 (0.18)^b	0.857
	n-6PUFA+LCn-3PUFA	0.46 (0.11)	0.29 (0.15)	-0.20 (0.18)^b	
IDL-TG	SFA+LCn-3PUFA	0.06 (0.02)	0.05 (0.02)	-0.00 (0.02)	0.033
	n-6PUFA+LCn-3PUFA	0.06 (0.02)	0.06 (0.02)	-0.02 (0.02)^b	
LDL-TG	SFA+LCn-3PUFA	0.12 (0.15)	0.12 (0.09)	0.02 (0.02)^b	0.042
	n-6PUFA+LCn-3PUFA	0.10 (0.06)	0.11 (0.03)	-0.01 (0.04)	
HDL-TG	SFA+LCn-3PUFA	0.04 (0.08)	0.05 (0.05)	0.01 (0.03)	0.117
	n-6PUFA+LCn-3PUFA	0.06 (0.05)	0.05 (0.04)	-0.01 (0.03)	
Total TG	SFA+LCn-3PUFA	0.70 (0.20)	0.54 (0.24)	-0.16 (0.24)^b	0.503
	n-6PUFA+LCn-3PUFA	0.67 (0.18)	0.49 (0.15)	-0.19 (0.28)^b	

Abbreviations: C, cholesterol; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LCn-3PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; n-6PUFA, omega-6 polyunsaturated fatty acid; SFA, saturated fatty acid; TG, triglycerides; VLDL, very-low-density lipoprotein. ^aP-values for comparison of change values between diets. ^bSignificant difference between pre and post intervention ($P < 0.05$). Data presented as median (interquartile range) for the SFA+LCn-3PUFA diet ($n = 14$; 3 males and 11 females) and the n-6PUFA+LCn-3PUFA diet ($n = 12$; 3 males and 9 females). Bold values in the delta-change column indicate significant difference between pre and post intervention ($P < 0.05$). Bold values in the P-value column indicate $P < 0.05$.

intermediate-density lipoprotein and LDL particles after intervention than subjects on the n-6PUFA+LCn-3PUFA. There was also an increase in cholesterol levels in HDL particles after consumption of both diets, although this increase was

significant only for subjects consuming the n-6PUFA+LCn-3PUFA diet ($P = 0.012$) (Table 1).

Total, large, medium and small VLDL particles were decreased after the consumption of both diets (n-SFA+LCn-3PUFA diet,

Table 2. Lipoprotein profiles (distribution in subclasses, concentration and size) of subjects at pre and post intervention

		Pre-intervention	Post-intervention	Δ Change	P-value ^a	P-value ^b
<i>Particle concentrations</i>						
VLDL-P (nmol/l)	SFA+LCn-3PUFA	23.93 (17.27)	13.85 (16.28)	-10.85 (9.53)	< 0.001	0.939
	n-6PUFA+LCn-3PUFA	26.03 (8.76)	13.63 (9.09)	-13.34 (12.53)	< 0.001	
Large VLDL-P (nmol/l)	SFA+LCn-3PUFA	0.73 (0.26)	0.55 (0.45)	-0.20 (0.33)	0.025	0.625
	n-6PUFA+LCn-3PUFA	0.77 (0.31)	0.57 (0.25)	-0.21 (0.24)	< 0.001	
Medium VLDL-P (nmol/l)	SFA+LCn-3PUFA	3.77 (2.36)	2.32 (1.90)	-1.44 (1.74)	< 0.001	0.979
	n-6PUFA+LCn-3PUFA	4.01 (1.19)	2.40 (1.09)	-1.80 (1.58)	< 0.001	
Small VLDL-P (nmol/l)	SFA+LCn-3PUFA	19.35 (14.74)	10.77 (14.01)	-9.33 (7.91)	< 0.001	0.898
	n-6PUFA+LCn-3PUFA	20.96 (7.46)	10.45 (6.67)	-11.28 (10.65)	< 0.001	
LDL-P (nmol/l)	SFA+LCn-3PUFA	725.96 (126.18)	739.01 (189.47)	39.61 (159.37)	0.173	0.292
	n-6PUFA+LCn-3PUFA	682.61 (210.32)	667.68 (79.54)	2.47 (146.53)	0.91	
Very large LDL-P (nmol/l)	SFA+LCn-3PUFA	107.87 (28.98)	108.69 (39.00)	7.13 (20.71)	0.119	0.341
	n-6PUFA+LCn-3PUFA	99.90 (27.19)	100.14 (15.63)	1.50 (14.08)	0.301	
Medium-large LDL-P (nmol/l)	SFA+LCn-3PUFA	243.37 (54.73)	249.17 (72.74)	21.51 (47.64)	0.011	0.173
	n-6PUFA+LCn-3PUFA	229.28 (66.87)	235.70 (42.65)	3.85 (32.93)	0.519	
Small LDL-P (nmol/l)	SFA+LCn-3PUFA	380.24 (88.79)	388.19 (100.45)	6.53 (88.01)	0.426	0.341
	n-6PUFA+LCn-3PUFA	352.99 (105.16)	328.79 (46.41)	-5.32 (90.16)	0.519	
HDL-P (μ mol/l)	SFA+LCn-3PUFA	24.44 (6.79)	27.13 (3.99)	1.40 (4.35)	0.173	1.000
	n-6PUFA+LCn-3PUFA	23.10 (6.43)	23.32 (9.35)	0.60 (1.97)	0.077	
Very large HDL-P (μ mol/l)	SFA+LCn-3PUFA	0.10 (0.07)	0.06 (0.06)	-0.04 (0.05)	0.011	0.738
	n-6PUFA+LCn-3PUFA	0.10 (0.05)	0.06 (0.05)	-0.05 (0.05)	< 0.001	
Medium-large HDL-P (μ mol/l)	SFA+LCn-3PUFA	8.11 (1.36)	8.95 (1.41)	0.56 (1.63)	0.058	1.000
	n-6PUFA+LCn-3PUFA	7.03 (2.52)	7.54 (3.71)	0.65 (1.06)	0.064	
Small HDL-P (μ mol/l)	SFA+LCn-3PUFA	16.53 (4.03)	18.00 (2.21)	0.95 (3.30)	0.217	0.817
	n-6PUFA+LCn-3PUFA	15.96 (4.26)	15.83 (6.21)	0.06 (1.26)	0.519	
<i>Lipoprotein particle size (nm)</i>						
VLDL-Z	SFA+LCn-3PUFA	42.17 (0.53)	42.48 (0.67)	0.27 (0.45)	0.042	0.662
	n-6PUFA+LCn-3PUFA	42.22 (0.62)	42.46 (0.80)	0.37 (0.46)	0.007	
LDL-Z	SFA+LCn-3PUFA	21.11 (0.10)	21.16 (0.15)	0.04 (0.08)	0.030	0.341
	n-6PUFA+LCn-3PUFA	21.11 (0.08)	21.16 (0.12)	0.06 (0.10)	0.027	
HDL-Z	SFA+LCn-3PUFA	8.23 (0.03)	8.24 (0.05)	0.00 (0.04)	0.670	0.662
	n-6PUFA+LCn-3PUFA	8.23 (0.01)	8.22 (0.04)	-0.01 (0.05)	0.970	

Abbreviations: HDL, high-density lipoprotein; LDL, intermediate-density lipoprotein; LCn-3PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; n-6PUFA, omega-6 polyunsaturated fatty acid; P, particle; SFA, saturated fatty acid; VLDL, very-low-density lipoprotein; Z, size (diameter in nm). ^aP-values for comparison between pre and post intervention. ^bP-values for comparison of change values between diets. Data presented as median (interquartile range) for the SFA+LCn-3PUFA diet ($n = 14$; 3 males and 11 females) and the n-6PUFA+LCn-3PUFA diet ($n = 12$; 3 males and 9 females). Bold values in delta-change and P-value (a) indicate significant difference for comparison between pre and post intervention ($P < 0.05$). Bold values for the P-value (b) column represent significant difference for comparison of values between diets.

$P < 0.001$, $P = 0.025$, $P < 0.001$ and $P < 0.001$; n-6PUFA+LCn-3PUFA diet, $P < 0.001$, respectively). A decrease was also observed in very large HDL particles after both diets (n-SFA+LCn-3PUFA diet, $P = 0.01$; n-6PUFA+LCn-3PUFA diet, $P < 0.001$, respectively), with no change in total HDL particle concentration and a tendency for increase in medium-large HDL particles after the SFA+LCn-3PUFA ($P = 0.058$) and the n-6PUFA+LCn-3PUFA ($P = 0.064$) diet. After the SFA+LCn-3PUFA, there was a significant increase in the concentration of medium-large LDL particles ($P = 0.011$). However, change in medium-large LDL was not significantly different between the two diets. Increases in VLDL and LDL particle size were not significantly different between both diets (Table 2). For all other parameters, there was no significant difference between diets.

DISCUSSION

The present intervention trial was designed to evaluate the effect of diets enriched with either SFA or n-6PUFA, and supplemented with LCn-3PUFA, on lipoprotein profiles as a secondary outcome. Both diets produced similar changes in lipoprotein profiles, suggesting that neither SFA nor n-6PUFA modulate LCn-3PUFA-induced changes in lipoprotein profiles. In addition, both diets increased LDL particle size and profile that has been associated with a reduced risk for atherosclerosis in epidemiological studies.

Subjects consuming either of the diets presented comparable reductions in total, large, medium and small VLDL particle concentrations, as well as in VLDL triglycerides, consistent with the decrease in total triglycerides and VLDL particle concentration following LCn-3PUFA supplementation observed in other studies.^{23,28,29} Decrease in plasma triglycerides and VLDL particles have been associated with a protective profile against atherosclerosis and cardiovascular disease risk.^{6,30} Supplementation with n-3PUFA has been shown to inhibit VLDL synthesis³¹ and secretion by the liver,³² improve VLDL clearance,³³ and enhance VLDL conversion to LDL.²⁹ One of the mechanisms by which LCn-3PUFA may act is the stimulation of lipoprotein lipase expression; resulting in triglyceride hydrolysis in chylomicrons and VLDL particles, forming lipoprotein remnants (small VLDL and intermediate-density lipoprotein particles). Lipoprotein remnants have more affinity to the LDL receptor (LDL-r),^{28,34} and compete with LDL particles for clearance.^{35,36} In addition, both LCn-3PUFA and SFA decrease expression of hepatic LDL-r,^{33,37} also explaining the increase in large LDL particle concentration after consumption of the SFA+LCn-3PUFA diet. N-6PUFA are also known to reduce circulating VLDL particle concentration, by increasing VLDL uptake and lipolysis.³⁸ The n-6PUFA lipolysis effect in VLDL may also explain the reduction in triglycerides in intermediate-density lipoprotein particles that occurred after the consumption of the n-6PUFA+LCn-3PUFA diet, but not after the SFA+LCn-3PUFA diet.

Despite the VLDL lowering ability of both n-6 and LCn-3PUFA, the n-6PUFA+LCn-3PUFA diet did not affect VLDL particle concentration and subclass distribution differently than the SFA+LCn-3PUFA diet, suggesting no increased benefit obtained in VLDL particle modulation by combining n-6 and LCn-3PUFA.

N-6PUFA have also been demonstrated to reduce plasma LDL particle concentration by increasing the fractional catabolism of apoB-100,³⁹ the main apolipoprotein in VLDL and LDL particles, and by increasing LDL-r transcription and synthesis.^{35,40} However, no significant change was observed in LDL particle concentration or distribution after the consumption of the n-6PUFA+LCn-3PUFA diet in our study, suggesting that LCn-3PUFA may have neutralized the LDL lowering effect of n-6PUFA.

The size of VLDL particles increased in both groups, conflicting with findings from Mostad *et al.*²¹ and Maki *et al.*,⁴¹ who observed a decrease in VLDL particle size in type II diabetes and hypercholesterolaemic patients, respectively, after supplementation with LCn-3PUFA compared to placebo (corn oil and soya oil, respectively). This may suggest that either both background dietary fats (n-6PUFA and SFA) suppress the ability of LCn-3PUFA to reduce VLDL size or LCn-3PUFA modulate VLDL size differently in healthy subjects.

After the consumption of both diets, there was an increase in LDL size and medium-large LDL particle concentration. Epidemiological studies suggest that serum LCn-3PUFA level is positively correlated with LDL particle size.^{7,42} Furthermore, intervention trials suggested an increase in LDL particle size,⁴³ especially when DHA is supplemented in the diet.¹³ The increase in medium-large LDL particle concentration is also consistent with previously published studies.^{14,22}

No change in total HDL particle concentration was observed, despite a decrease in very large HDL particle concentration. Indeed, there was a trend for an increase in medium-large HDL particle concentration after both diets, which counter-balanced the decrease of very large HDL particles. This is consistent with previous epidemiological and interventional studies. In epidemiological studies, a positive correlation between dietary LCn-3PUFA and serum large HDL particle concentration was observed.^{7,44} In interventional trials, an increase in large HDL particle concentration was observed in the plasma of mildly and hypercholesterolaemic subjects during a DHA supplementation trial,^{13,14} and no change in plasma small, medium or large HDL particles after supplementation with EPA.¹⁷ The very large HDL particles (10.5–13.5 nm) in the present intervention are just a small proportion of the total HDL particles and even a small change in magnitude may result in a significant change. Nevertheless, medium-large HDL particles (8.5–10.5 nm) represent mature HDL particles and therefore active in the reverse transport of cholesterol, conferring a profile protective against atherosclerosis risk.

The decrease in very large HDL particles after consumption of both diets is consistent with an increase in the activity of cholesteryl ester transfer protein caused by LCn-3PUFA supplementation, especially DHA.³⁷ An increase in cholesteryl ester transfer protein activity consequently increases the exchange of cholesterol from HDL particles for triglycerides from VLDL particles, improving the reverse cholesterol transport and facilitating the conversion of VLDL to LDL. This mechanism may also be responsible for the concomitant decrease in VLDL particles and increase in LDL triglycerides. Triglyceride-rich HDL particles have their triglycerides hydrolysed by hepatic lipase, which may explain the shift in the subclass distribution of HDL particles.⁴⁵

Comparison between the different studies performed on lipoprotein profiles is limited by the lack of standardization between the different analytical methods used for the determination of lipoprotein profiles,⁴⁶ although the gap between methods has been closing with recent studies.⁴⁷ Furthermore, the literature on the classification of lipoprotein subclasses according to their diameter and density is heterogeneous. The method used was

limited by the fact that intermediate-density particles were not measured separately,²⁵ but were combined with very large LDL particles. However, large and small LDL subclasses and VLDL particles seem to be of more importance to atherosclerosis management.¹ Another limitation in the discussion about the effect of LCn-3PUFA on lipoprotein profiles is the difference in composition and dose of LCn-3PUFA used across different studies, as well as the lack of dietary information. In addition, sample size and power were calculated for determining change in plasma triglycerides and not specifically for determining changes in lipoprotein profiles. Thus, the sample size may have been small to assess differences in lipoprotein profiles, as the changes are small in magnitude, especially for HDL profile. The results may also have been confounded by sex- and age-dependent effects, as a mixed population was assessed. Moreover, it is possible that a small increase in LCn-3PUFA in tissues may produce a desirable effect on lipoprotein profiles that is not improved with a further increase in LCn-3PUFA. In which case, the high LCn-3PUFA dose may have masked the effects of the background dietary fat consumed.

In conclusion, a background diet rich in saturated fatty acids versus n-6PUFA did not modulate the effect of LCn-3PUFA on lipoprotein profiles, despite the enhanced LCn-3PUFA incorporation into plasma and erythrocyte lipids with consumption of a diet rich in saturated fatty acids compared to n-6PUFA.²⁴ Further investigation of the interaction of LCn-3PUFA with saturated fats is warranted, to clarify the role of LCn-3PUFA status in modulating dietary SFA-induced changes in circulating lipoproteins. However, the changes to lipoprotein profiles observed suggest an improvement towards a less atherogenic profile after intervention with both diets, supporting the hypothesis that a diet rich in saturated fatty acids does not modulate cardiovascular risk factors differently than a diet rich in n-6PUFA when an adequate amount of LCn-3PUFA is consumed. Furthermore, sex differences, as well as age, dose and intervention length, should be considered in future studies.

CONFLICT OF INTEREST

NA is a stock owner of Biosfer Teslab. The remaining authors declare no conflict of interest.

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REFERENCES

- 1 Carmena R, Duriez P, Fruchart J-C. Atherogenic lipoprotein particles in atherosclerosis. *Circulation* 2004; **109**(suppl 1): III2–III7.
- 2 Mackey RH, McTigue KM, Chang YF, Barinas-Mitchell E, Evans RW, Tinker LF *et al.* Lipoprotein particles and size, total and high molecular weight adiponectin, and leptin in relation to incident coronary heart disease among severely obese postmenopausal women: The Women's Health Initiative Observational Study. *BBA Clin* 2015; **3**: 243–250.
- 3 Akinkuolie AO, Paynter NP, Padmanabhan L, Mora S. High-density lipoprotein particle subclass heterogeneity and incident coronary heart disease. *Circ Cardiovasc Qual Outcomes* 2014; **7**: 55–63.
- 4 Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation* 2009; **119**: 931–939.
- 5 Mackey RH, Kuller LH, Sutton-Tyrrell K, Evans RW, Holubkov R, Matthews KA. Lipoprotein subclasses and coronary artery calcium in postmenopausal women from the healthy women study. *Am J Cardiol* 2002; **90**: 711–761.

- 6 Cromwell WC, Otvos JD, Keyes MJ, Pencina MJ, Sullivan L, Vasan RS *et al*. LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study—implications for LDL management. *J Clin Lipidol* 2007; **1**: 583–592.
- 7 Bogl LH, Pietiläinen KH, Rissanen A, Kangas AJ, Soininen P, Rose RJ *et al*. Association between habitual dietary intake and lipoprotein subclass profile in healthy young adults. *Nutr Metab Cardiovasc Dis* 2013; **23**: 1071–1078.
- 8 Ahmed HM, Blaha MJ, Nasir K, Rivera JJ, Blumenthal RS. Effects of physical activity on cardiovascular disease. *Am J Cardiol* 2012; **109**: 288–295.
- 9 Rizzo EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. *JAMA* 2012; **308**: 1024–1033.
- 10 Eslick GD, Howe PRC, Smith C, Priest R, Bensoussan A. Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. *Int J Cardiol* 2009; **136**: 4–16.
- 11 Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem Pharmacol* 2009; **77**: 937–946.
- 12 Phang M, Lincz LF, Garg ML. Eicosapentaenoic and docosahexaenoic acid supplementations reduce platelet aggregation and hemostatic markers differentially in men and women. *J Nutr* 2013; **143**: 457–463.
- 13 Mori TA, Burke V, Puddey IB, Watts GF, O'Neal DN, Best JD *et al*. Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr* 2000; **71**: 1085–1094.
- 14 Neff LM, Culiner J, Cunningham-Rundles S, Seidman C, Meehan D, Maturi J *et al*. Algal docosahexaenoic acid affects plasma lipoprotein particle size distribution in overweight and obese adults. *J Nutr* 2011; **141**: 207–213.
- 15 Kelley DS, Siegel D, Vemuri M, Mackey BE. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr* 2007; **86**: 324–333.
- 16 Erkkilä AT, Schwab US, Lehto S, de Mello VD, Kangas AJ, Soininen P *et al*. Effect of fatty and lean fish intake on lipoprotein subclasses in subjects with coronary heart disease: a controlled trial. *J Clin Lipidol* 2014; **8**: 126–133.
- 17 Bays HE, Braeckman RA, Ballantyne CM, Kastelein JJ, Otvos JD, Stirtan WG *et al*. Icosapent ethyl, a pure EPA omega-3 fatty acid: effects on lipoprotein particle concentration and size in patients with very high triglyceride levels (the MARINE study). *J Clin Lipidol* 2012; **6**: 565–572.
- 18 Mostad IL, Bjerre KS, Bjorgaas MR, Lydersen S, Grill V. Effects of n3 fatty acids in subjects with type 2 diabetes: reduction of insulin sensitivity and time-dependent alteration from carbohydrate to fat oxidation. *Am J Clin Nutr* 2006; **84**: 540–550.
- 19 Satoh N, Shimatsu A, Kotani K, Sakane N, Yamada K, Suganami T *et al*. Purified eicosapentaenoic acid reduces small dense LDL, remnant lipoprotein particles, and C-reactive protein in metabolic syndrome. *Diabetes Care* 2007; **30**: 144–146.
- 20 Tani S, Nagao K, Matsumoto M, Hirayama A. Highly purified eicosapentaenoic acid may increase low-density lipoprotein particle size by improving triglyceride metabolism in patients with hypertriglyceridemia. *Circ J* 2013; **77**: 2349–2357.
- 21 Mostad IL, Bjerre KS, Lydersen S, Grill V. Effects of marine n-3 fatty acid supplementation on lipoprotein subclasses measured by nuclear magnetic resonance in subjects with type II diabetes. *Eur J Clin Nutr* 2007; **62**: 419–429.
- 22 Oelrich B, Dewell A, Gardner CD. Effect of fish oil supplementation on serum triglycerides, LDL cholesterol and LDL subfractions in hypertriglyceridemic adults. *Nutr Metab Cardiovasc Dis* 2013; **23**: 350–357.
- 23 Rivelles AA, Maffettone A, Vessby B, Uusitupa M, Hermansen K, Berglund L *et al*. Effects of dietary saturated, monounsaturated and n-3 fatty acids on fasting lipoproteins, LDL size and post-prandial lipid metabolism in healthy subjects. *Atherosclerosis* 2003; **167**: 149–158.
- 24 Dias CB, Wood LG, Garg ML. Effects of dietary saturated and n-6 polyunsaturated fatty acids on the incorporation of long chain n-3 polyunsaturated fatty acids into blood lipids. *Eur J Clin Nutr* 2016; **70**: 812–818.
- 25 Mallol R, Amigo N, Rodriguez MA, Heras M, Vinaixa M, Plana N *et al*. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. *J Lipid Res* 2015; **56**: 737–746.
- 26 Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med* 2006; **26**: 847–870.
- 27 Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML. Increased plasma fatty acid concentrations after respiratory exacerbations are associated with elevated oxidative stress in cystic fibrosis patients. *Am J Clin Nutr* 2002; **75**: 668–675.
- 28 Mori TA, Vandongen R, Beilin LJ, Burke V, Morris J, Ritchie J. Effects of varying dietary fat, fish, and fish oils on blood lipids in a randomized controlled trial in men at risk of heart disease. *Am J Clin Nutr* 1994; **59**: 1060–1068.
- 29 Chan DC, Watts GF, Mori TA, Barrett PHR, Redgrave TG, Beilin LJ. Randomized controlled trial of the effect of n-3 fatty acid supplementation on the metabolism of apolipoprotein B-100 and chylomicron remnants in men with visceral obesity. *Am J Clin Nutr* 2003; **77**: 300–307.
- 30 Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. *Lancet* 2014; **384**: 626–635.
- 31 Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996; **37**: 907–925.
- 32 López-Soldado I, Avella M, Botham KM. Suppression of VLDL secretion by cultured hepatocytes incubated with chylomicron remnants enriched in n-3 polyunsaturated fatty acids is regulated by hepatic nuclear factor-4a. *Biochim Biophys Acta* 2009; **1791**: 1181–1189.
- 33 Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. *J Nutr* 2005; **135**: 2075–2078.
- 34 Griffin BA. The effect of n-3 fatty acids on low density lipoprotein subfractions. *Lipids* 2001; **36**: S91–S97.
- 35 Jackson KG, Maitin V, Leake DS, Yaqoob P, Williams CM. Saturated fat-induced changes in Sf 60–400 particle composition reduces uptake of LDL by HepG2 cells. *J Lipid Res* 2006; **47**: 393–403.
- 36 Olano-Martin E, Anil E, Caslake MJ, Packard CJ, Bedford D, Stewart G *et al*. Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. *Atherosclerosis* 2010; **209**: 104–110.
- 37 Ishida T, Ohta M, Nakakuki M, Kami H, Uchiyama R, Kawano H *et al*. Distinct regulation of plasma LDL cholesterol by eicosapentaenoic acid and docosahexaenoic acid in high fat diet-fed hamsters: participation of cholesterol ester transfer protein and LDL receptor. *Prostaglandins Leukot Essent Fatty Acids* 2013; **88**: 281–288.
- 38 van Schalkwijk DB, Pasman WJ, Hendriks HFJ, Verheij ER, Rubingh CM, van Bochove K *et al*. Dietary medium chain fatty acid supplementation leads to reduced VLDL lipolysis and uptake rates in comparison to linoleic acid supplementation. *PLoS ONE* 2014; **9**: e100376.
- 39 Ooi E, Watts G, Ng T, Barrett P. Effect of dietary fatty acids on human lipoprotein metabolism: a comprehensive update. *Nutrients* 2015; **7**: 4416.
- 40 Mustad VA, Ellsworth JL, Cooper AD, Kris-Etherton PM, Etherton TD. Dietary linoleic acid increases and palmitic acid decreases hepatic LDL receptor protein and mRNA abundance in young pigs. *J Lipids Res* 1996; **37**: 2310–2323.
- 41 Maki KC, Lawless AL, Kelley KM, Dicklin MR, Kaden VN, Schild AL *et al*. Effects of prescription omega-3-acid ethyl esters on fasting lipid profile in subjects with primary hypercholesterolemia. *J Cardiovasc Pharmacol* 2011; **57**: 489–494.
- 42 Mantyselka P, Niskanen L, Kautiainen H, Saltevo J, Wurtz P, Soininen P *et al*. Cross-sectional and longitudinal associations of circulating omega-3 and omega-6 fatty acids with lipoprotein particle concentrations and sizes: population-based cohort study with 6-year follow-up. *Lipids Health Dis* 2014; **13**: 28.
- 43 Lee MW, Park JK, Hong JW, Kim KJ, Shin DY, Ahn CW *et al*. Beneficial effects of omega-3 fatty acids on low density lipoprotein particle size in patients with type 2 diabetes already under statin therapy. *Diabetes Metab J* 2013; **37**: 207–211.
- 44 Annuzzi G, Rivelles AA, Wang H, Patti L, Vaccaro O, Riccardi G *et al*. Lipoprotein subfractions and dietary intake of n-3 fatty acid: the Genetics of Coronary Artery Disease in Alaska Natives study. *Am J Clin Nutr* 2012; **95**: 1315–1322.
- 45 Kuang Y-L, Eric Paulson K, Lichtenstein AH, Lamon-Fava S. Regulation of the expression of key genes involved in HDL metabolism by unsaturated fatty acids. *Br J Nutr* 2012; **108**: 1351–1359.
- 46 Ensign W, Hill N, Heward CB. Disparate LDL phenotypic classification among 4 different methods assessing LDL particle characteristics. *Clin Chem* 2006; **52**: 1722–1727.
- 47 Sninsky JJ, Rowland CM, Baca AM, Caulfield MP, Superko HR. Classification of LDL phenotypes by 4 methods of determining lipoprotein particle size. *J Invest Med* 2013; **61**: 942–949.

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